CORRECTED VERSION

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 021081PC/KF	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU2003/001552	International Filing Date *(day/month/year)* 20 November 2003	Priority Date *(day/month/year)* 20 November 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] G01N 33/53, 35/00

Applicant

BIO-MOLECULAR HOLDINGS PTY LIMITED et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
21 June 2004	16 March 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **RAJEEV DESHMUKH** Telephone No. (02) 6283 2145

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001552

I. Basis of the report

1. With regard to the elements of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1, 3–9,** as originally filed,
  pages , filed with the demand,
  pages **2,** received on **3 March 2005** with the letter of **3 March 2005**
- [X] the claims, pages , as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages **10–12,** received on **3 March 2005** with the letter of **3 March 2005**
- [X] the drawings, pages **1/1,** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [ ] the sequence listing part of the description:
  pages , as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)):**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1–27	YES
	Claims	NO
Inventive step (IS)	Claims 1–27	YES
	Claims	NO
Industrial applicability (IA)	Claims 1–27	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

WO 2001/067102 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 13 September 2001—Abstract; page 4, lines 16-25; page 5, lines 14, 15; page 6, line 1 - page 7, line 4; page 24, line 4. There is no disclosure of binding a first molecular entity to a second molecular entity, the latter being located at an attachment zone. In particular there is no suggestion of attaching chitinous material at a particular zone of a centrifuge.

WO 1995/031731 A (GAMMA BIOLOGICALS, INC) 23 November 1995—Abstract; page 3, lines 13-15, lines 25-31. The particles—while being contained within a centrifuge tube—do not, however, constitute an attachment zone on the surface of the centrifuge tube.

NEW CITATION: US 3763374 A (TIFFANY et al.) 2 October 1973. This document discloses a rotor having a plurality of sample analysis cuvettes from which fluorometric and photometric measurements can be made but from *different* portions of the cuvette. There is no disclosure or suggestion of an attachment zone in the cuvettes where the binding partner interaction can be measured.

NOVELTY (N), INVENTIVE STEP (IS), INDUSTRIAL APPLICABILITY (IA)

None of the cited documents discloses or (individually or in an obvious combination) suggests the invention as claimed in claims 1–27 wherein an attachment zone is present on the internal surface of the centrifuge tube. Therefore the claimed invention is novel, involves an inventive step, and is industrially applicable.

SUMMARY OF THE INVENTION

In a first embodiment of the invention, there is provided a device for measuring the binding of a first partner in an interaction to a second partner in said interaction, wherein said interaction partners are molecular entities, said device comprising:

a) an opaque temperature-controlled chamber having a rotor therein, said rotor having at or near the periphery of the rotor and attached thereto at least one radially positioned transparent reaction well, said reaction well having on an upper surface thereof an aperture for the addition of reagents to the reaction well, said reaction well further including on an internal surface thereof at the end closest the axis of said rotor at least one attachment zone for said second interaction partner;

b) a stationary system for detecting light emitted or absorbed by said first interaction partner or an indicator molecule bound thereto; and

c) means for controlling the temperature of said chamber and the operation of said rotor.

In a second embodiment, the invention provides a method of measuring the binding of a first partner in an interaction to a second partner in said interaction, wherein said interaction partners are molecular entities, said method comprising the steps of:

a) delivering a quantity of second interaction partner to a reaction well of a device according to the first embodiment for attachment of said second interaction partner to an attachment zone of said reaction well;

b) combining a quantity of first interaction partner with said second interaction partner in said reaction well and incubating said mixture at a temperature and for a time to allow binding of said first interaction partner to said second interaction partner;

c) rotating said device rotor at a speed which displaces the mixture formed in step (b) away from said attachment zone; and

d) measuring the amount of said first interaction partner bound to said second interaction partner via the fluorescence or absorbance of said first interaction partner or an indicator molecule bound thereto.

Other embodiments of the invention will become apparent from a reading of the detailed description below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a semi schematic representation of a rotor of a device according to the

CLAIMS

1. A device for measuring the binding of a first partner in an interaction to a second partner in said interaction, wherein said interaction partners are molecular entities, said device comprising:

a) an opaque temperature-controlled chamber having a centrifuge rotor therein, said rotor having at or near the periphery of the rotor and attached thereto at least one radially positioned transparent reaction well, said reaction well having on an upper surface thereof an aperture for the addition of reagents to the reaction well, said reaction well further including on an internal surface thereof at the end closest the axis of said rotor at least one attachment zone for said second interaction partner;

b) a stationary system for detecting light emitted or absorbed by said first interaction partner or an indicator molecule bound thereto; and

c) means for controlling the temperature of said chamber and the operation of said rotor.

2. The device of claim 1, wherein said chamber has a lid or sealable opening to allow loading of reaction wells.

3. The device of claim 1, wherein said temperature control is effected by providing a heater linked to a temperature sensor so that a set temperature can be maintained.

4. The device of claim 1, wherein said temperature control includes a cooling system.

5. The device of claim 1, wherein said rotor comprises a flat disc of a plastic or metal material having said at least one reaction well fitted therein.

6. The device of claim 1, wherein said rotor has from 1 to 96 reaction wells.

7. The device of claim 1, wherein said at least one reaction well is manufactured from polypropylene or polycarbonate.

8. The device of claim 1, wherein said at least one reaction well is cylindrical or a rectangular prism.

9. The device of claim 1, wherein said at least one reaction well is angled upwards toward the periphery of the rotor.

10. The device of claim 1, wherein said attachment zone is provided by spotting the second interaction partner onto said internal surface of the reaction well.

11. The device of claim 1, wherein said attachment zone is provided by way of said second interaction partner being linked to a magnetic particle which is held in the attachment zone by a magnet.

12. The device of claim 1, wherein said attachment zone is circular with a diameter of 50 μm to 3 mm.

13. The device of claim 1, wherein said drive means is a direct-coupled AC motor, a DC motor or stepper motor with the motor external to the chamber.

14. The device of claim 1, wherein said light source is an LED, a laser light source or a halogen lamp.

15. The device of claim 1, wherein said device further includes a computer for controlling an operation selected from the group consisting of rotor speed, chamber temperature, time and temperature for annealing and polymerization steps when the binding assay is a hybridization, rotor braking, rotor vibration, and data processing.

16. A method of measuring the binding of a first partner in an interaction to a second partner in said interaction, wherein said interaction partners are molecular entities, said method comprising the steps of:

a) delivering a quantity of second interaction partner to a reaction well of a device according to claim 1 for attachment of said second interaction partner to an attachment zone of said reaction well;

b) combining a quantity of first interaction partner with said second interaction partner in said reaction well and incubating said mixture at a temperature and for a time to allow binding of said first interaction partner to said second interaction partner;

c) rotating said device rotor at a speed which displaces the mixture formed in step (b) away from said attachment zone; and

d) measuring the amount of said first interaction partner bound to said second interaction partner via the fluorescence or absorbance of said first interaction partner or an indicator molecule bound thereto.

17. The method of claim 16, wherein said first and second interaction partners are respectively selected from the group consisting of the following combinations: an antibody and an antigen; an antigen and an antibody; an enzyme and a substrate; an oligopeptide and a

protein; a hormone and a receptor; an effector molecule and a receptor; a nucleic acid and a nucleic acid; an oligonucleotide and a nucleic acid; and, a synthetic organic compound and a protein

18. The method of claim 16, wherein said first and second interaction partners are delivered as solutions containing other components selected from the group consisting of buffers, salts, DNA or RNA polymerization reagents including a polymerase, and a blocking reagent.

19. The method of claim 16, wherein step (b) is carried out with the rotor rotating at a speed of up to 500 rpm.

20. The method of claim 16, wherein step (c) is carried out with the rotor rotating at a speed of greater than 500 rpm.

21. The method of claim 16, wherein said first interaction partner has a fluorophore bound thereto selected from the group consisting of FAM, JOE, ROX, TAMRA, Cy5, Cy3, Cy5.5, and VIC.

22. The method of claim 16, wherein said first interaction partner has a Dabcyl or BH quencher absorbent group bound thereto.

23. The method of claim 16, wherein said indicator molecule is an intercalating dye.

24. The method of claim 23, wherein said intercalating dye is Sybr green.

25. The method of claim 16, wherein said the indicator molecule is a derivatised antibody.

26. The method of claim 16, wherein in step (d) said absorbance or fluorescence is measured with the rotor rotating at a speed of at least 500 rpm.

27. The method of claim 16, wherein said at least one reaction well has multiple attachment zones and measurement of the amount of said first interaction partner bound to said second interaction partner via the fluorescence or absorbance of said first interaction partner or an indicator molecule bound thereto is by way of multiple detectors.